Influence of starter culture and ripening temperature on survival of *L. monocytogenes* in traditional Portuguese dry sausages

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Background

Linguiça is a Portuguese popular ready-to-eat dry fermented sausage. Despite the high diversity of sausages produced in Portugal, all of them are made of diced pork meat macerated with water, wine, salt, garlic, pepper, and undergo processes of maturation, smoking and drying. During fermentation and drying of sausages, *L. monocytogenes* tends to decrease considerably. However, despite the various hurdles in the dry sausage manufacturing process of *linguiça*, this foodborne pathogen can still survive and be detected in the final product. Factors that may affect the growth or survival of foodborne pathogens in *linguiça* include water activity (aw), pH, temperature, use of starter cultures and use of ingredients with antimicrobial activity (e.g., garlic, smoke).

Aim:

The present study evaluated the influence of the addition of a commercial starter culture and the ripening temperature (10º C and 18ºC) on the survival of a *L. monocytogenes* strain experimentally inoculated in *linguiça*.

Methodology

Sausage manufacture

Diced raw pork meat was mixed with salt (20.0 g/kg), dry garlic (4.5 g/kg), sweet pepper (12.5 g/kg), laurel (0.5 g/kg), dextrose (10.0 g/kg), a mix of red/white wine (410 ml/kg) and water (410 ml/kg), and inoculated with *L. monocytogenes* to reach a concentration of ~5 $\log_{10}$/g in the batter. Additionally, commercial starter culture (5 $\log_{10}$/g) was added to one batch. The batter was macerated for 3 days at 4ºC. After stuffing into natural pork casings, sausages were hung vertically in a climate controlled chamber for ripening, at 10ºC or 18ºC with 80% relative humidity (RH) during ten days. *L. monocytogenes*-inoculated sausages were manufactured for four treatments:

(1) Without starter and ripened at 10ºC
(2) With starter and ripened at 10ºC
(3) Without starter and ripened at 18ºC
(4) With starter and ripened at 18ºC

Sampling

For the microbiological and physicochemical analyses, the samples from each batch were collected along production in the following sampling points: meat mixed with ingredients (day 0), macerated meat before stuffing (day 3) and during the ripening process (days 5, 7, 9, 11, 12, 13 and 14).

Microbiological and physicochemical analyses

L. Monocytogenes was enumerated according to the ISO 11290-2:1998/Amd. 1:2004(E) procedure, and using Oxoid Chromogenic Listeria Agar (OCLA, Oxoid). Lactic acid bacteria (LAB) counting was performed on Man, Rogosa and Sharpe (MRS) agar (Liofilchem, Italy) overlaid with 5 mL agar 0.8 %. The pH was measured directly in the centre of the samples with a pH-meter HI8424 (Hanna Instruments, Portugal) while water activity (Aw) was measured using a Dew Point Water Activity Meter 4E (AquaLab, USA).

Statistical analysis

The effects of addition of starter culture and ripening temperature on the survival of *L. monocytogenes* in *linguiça* was assessed by fitting a three-parameter Weibull-based regression model to the data:

$$\log N_{jk} = \log N_0 - \left(\frac{t}{\chi_k}\right)^{\beta_\rho} + \varepsilon_{jk}$$

$$Ln\,\beta_{jk} = a_1 Starter_j + a_2 T_k + a_3 Starter_j T_k$$

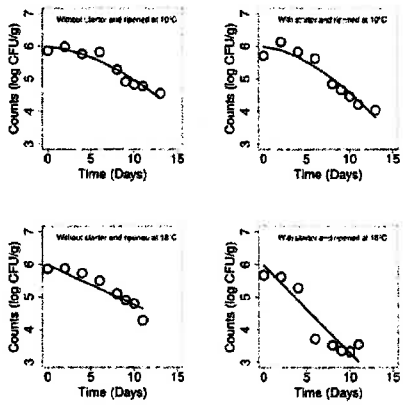
$$Ln\,\chi_k = bT_k$$

where:

Starter: a coded variable defining the use or non-use of starter culture (j=2)
T: ripening temperature, 10ºC or 18ºC (k=2)
t: time (day)
log N: concentration of *L. monocytogenes* in sausages during ripening (log CFU/g)
log N_0: initial concentration of *L. monocytogenes* (t=0)
χ and **β**: scale and shape parameter of the Weibull decay function, respectively
ε: normally-distributed residuals

Results

During the three days of maceration, there was no significant change in the counts of LAB or *L. monocytogenes*. However, during ripening, in the four treatments, LAB counts increased (not shown) while *L. monocytogenes* decreased steadily. The Weibull-based model demonstrated that both the use of starter culture and the ripening temperature ($p<0.05$) affected the concavity (shape parameter β) of the survival curves, meaning that a faster decay in *L. monocytogenes* is expected in *linguiça* formulated with commercial starter culture and ripened at higher temperature (18ºC). While a higher ripening temperature ($p=0.04$) reduced the time to reach the first decimal reduction (scale parameter χ) in *L. monocytogenes* in *linguiça*, the use of the commercial starter had no effect on such lethality parameter.



In the batches without starter culture, by the 10th day of ripening, *L. monocytogenes* population was reduced in 1.10 log CFU/g at 10ºC and 1.30 log CFU/g at 18ºC of ripening temperature. With the addition of a commercial starter culture, the pathogen was further reduced in 1.60 and 2.55 log CFU/g when sausages were ripened at 10ºC and 18ºC, respectively.

Conclusion

Since separate microbiological survey investigations in *linguiça* processing plants have pointed out the recovery of *L. monocytogenes* in the final product in concentrations normally lower than 50 CFU/g (1.70 log CFU/g), the use of starter cultures should be contemplated as an effective hurdle that can further bring down such levels of *L. monocytogenes*. As a next step, research should be directed towards the preparation of a tailor-made culture.

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